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<b>(21) International Application Number:</b> PCT/US00/05467 <b>(22) International Filing Date:</b> 3 March 2000 (03.03.00) <b>(30) Priority Data:</b> 9904939.7 5 March 1999 (05.03.99) GB <b>(71) Applicant (for all designated States except US):</b> E.I. DU PONT DE NEMOURS AND COMPANY [US/US]; 1007 Market Street, Wilmington, DE 19898 (US). <b>(72) Inventor; and</b> <b>(75) Inventor/Applicant (for US only):</b> GODDARD, Simon, James [GB/GB]; 7 Woodcock Close, Impington, Cambridgeshire CB4 9LD (GB). <b>(74) Agent:</b> BEARDELL, Lori, Y.; E.I. Du Pont de Nemours and Company, Legal Patent Records Center, 1007 Market Street, Wilmington, DE 19898 (US).		<b>(81) Designated States:</b> AE, AL, AU, BA, BB, BG, BR, CA, CN, CR, CU, CZ, DM, EE, GD, GE, HR, HU, ID, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, US, UZ, VN, YU, ZA, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> POLYMER COMPOSITIONS  <b>(57) Abstract</b>  The present invention is directed to a composition comprising a first polymer comprising arabinosyl-substituted (1-4)- $\beta$ -xylopyranosyl units cross-linked to a second polymer comprising D-galacturonic acid units. A composition comprising co-gelled first and second polymers, the first polymer comprising arabinosyl-substituted (1-4)- $\beta$ -xylopyranosyl units and the second polymer comprising D-galacturonic acid units is also embodied by the present invention. Products comprising the foregoing compositions are also contemplated by the present invention.		

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## POLYMER COMPOSITIONS

This application claims the benefit of British Application No. 9904939.7, filed March 5, 1999.

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FIELD OF THE INVENTION

The present invention relates to polymer compositions, and in particular to polymer compositions comprising a first polymer comprising arabinosyl-substituted (1-4)- $\beta$ -xylopyranosyl units cross-linked to a second polymer comprising D-galacturonic acid units. The polymer compositions may be vegetable gels and they have a wide variety of uses in the food and medical industries and in agriculture.

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BACKGROUND TO THE INVENTION

The term "hemicellulose" is a term of art used to embrace non-cellulosic, non-starch plant polysaccharides. The term therefore embraces *inter alia* pentosans, pectins and gums.

Some hemicelluloses are suitable as substrates for oxidative gelation ("gelling hemicelluloses"): such hemicelluloses often have substituents with phenolic groups which are cross-linkable with certain oxidizing agents.

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Arabinoxylan and pectin constitute two particularly important classes of hemicellulose. Arabinoxylans consist predominantly of the pentoses arabinose and xylose, and are therefore often classified as pentosans. However, in many cases hexoses and hexuronic acid are present as minor constituents, and therefore they may also be referred to descriptively as heteroxylans.

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The arabinoxylan molecule consists of a linear backbone of (1-4)- $\beta$ -xylopyranosyl units, to which substituents are attached through O2 and O3 atoms of the xylosyl residues. The major substituents are single  $\alpha$ -L-arabinofuranosyl residues. Single  $\alpha$ -D-glucuronopyranosyl residues and their 4-O-methyl ethers are also common substituents.

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Arabinoxylan preparations are usually heterogeneous with respect to the ratio of xylose to arabinose (i.e., the degree of substitution) and in the pattern of substitution of the arabinosyl units along the (1-4)- $\beta$ -xylan backbone.

Phenolic acid (including ferulic acid) and acetyl substituents may occur at intervals along the arabinoxylan chains. These substituents may have an effect on the solubility of the arabinoxylan. They render the arabinoxylan oxidatively cross-linkable to produce viscous solutions or gels *via* their phenolic substituents (referred to herein as "gelling arabinoxylans"). Arabinoxylan preparations bearing phenolic (e.g., ferulic acid) substituents are referred to herein as "AXF", while those bearing acetyl substituents are designated "AXA". Similarly, preparations bearing both phenolic (e.g., ferulic acid) and acetyl substituents are hereinafter abbreviated to the designation "AXFA". Arabinoxylan preparations having few phenolic (e.g., ferulic acid) substituents are designated "AX": when

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the degree of substitution falls below that required for oxidative gelation, the arabinoxylan is designated a "non-gelling arabinoxylan" (a term which therefore embraces AX and AXA).

Pectins constitute another important class of hemicelluloses. As used herein and unless otherwise indicated, the term "pectin" is used *sensu lato* to define hemicellulose polymers rich in D-galacturonic acid. Many (but not all) are cell wall components. The term "pectin" is also used herein *sensu stricto* to define the so-called "true pectins", which are characterized by the presence of an *O*-( $\alpha$ -D-galacturonopyranosyl)-(1-2)-L-rhamnopyranosyl linkage within the molecule.

The pectins may be subcategorized on the basis of their structural complexity. At one extreme are "simple pectins", which are galacturonans. At the other extreme are "complex pectins" exemplified by rhamnogalacturonan II, which contains at least 10 different monosaccharide components in the main chain or as components of branches. Pectins of intermediate complexity (herein referred to as "mesocomplex pectins" contain alternate rhamnose and galacturonic acid units, while others have branches of glucuronic acid linked to galacturonic acid.

Complex and mesocomplex pectins are made up of "smooth" regions (based on linear homogalacturonan) and "hairy" regions corresponding to the rhamnogalacturonan backbone with side-branches of varying length.

Certain pectins (for example, pectins obtainable from representatives of the plant family *Chenopodiaceae*, which include beets (e.g., sugar beet), spinach and mangelwurzel) are substituted to some extent with substituents derived from carboxylic acids (usually substituted cinnamic acids) containing phenolic groups. Such pectins (which include feruloylated pectins) may be oxidatively cross-linked to produce viscous solutions or gels *via* their phenolic substituents. This can be achieved by powerful oxidants (e.g., persulfate - see J.-F. Thibault *et alia*, in The Chemistry and Technology of Pectin, Academic Press 1991, Chapter 7, pages 119-133) or a combination of peroxidase and hydrogen peroxide (see Thibault *et alia*, *ibidem*). FR 2 545 101 A1 also describes the gelling of beet pectins using an oxidant (e.g., hydrogen peroxide) and an enzyme (peroxidase). Such pectins are referred to herein as "gelling pectins". Thus, gelling pectins may be subject to "oxidative gelation" (as herein defined).

Sugar beet pectin is especially rich in arabinan. Arabinan contains  $\beta$ -1,5-linked arabinose in the backbone with  $\alpha$ -(1 $\rightarrow$ 3) or  $\alpha$ -(1 $\rightarrow$ 2)-linked arabinose residues, whereas arabinogalactan contains  $\beta$ -1,4-linked galactose in the backbone, with  $\alpha$ -(1 $\rightarrow$ 3) or  $\alpha$ -(1 $\rightarrow$ 2) linked arabinose residues. Ferulyl substituents are linked to the arabinose and/or the galactose in the arabinan and arabinogalactan side-branches of the rhamnogalacturonan part. The "ferulic acid" content varies according to the extraction method, but is often about 0.6%.

Beet pectins obtained by processes which partially remove arabinose residues may exhibit improved gelling properties. Thus, procedures involving mild acid treatment and/or

treatment with an  $\alpha$ -arabinofuranosidase will improve the gelling properties of the pectin (see F. Guillon and J.-F. Thibault, *ibidem*). Such pectins are hereinafter referred to as "treated pectins".

#### Phenolic acids

5        The phenolic acids (chiefly ferulic and *p*-coumaric acids) are common in cell walls from cereal grains and have also been detected in barley husks and embryo. They may be attached to barley storage proteins and are found in starchy endosperm cell walls and in the aleurone layer. The phenolic acids (e.g., ferulic acid) may be associated with polysaccharides (such as hemicelluloses, e.g., arabinoxylans), where they may be cross-  
10       linkable by oxidative gelation (see *infra*). The phenolic aldehydes *p*-hydroxybenzaldehyde, vanillin and syringaldehyde have been identified in cell walls of grasses and are apparently linked at their phenolic groups.

#### Oxidative gelation, gelling hemicelluloses and hemicellulose gels

15       Aqueous extracts of several different types of hemicelluloses are known to form gels (or viscous liquids) when treated with certain oxidizing agents. For example, it has long been known that certain flour extracts (e.g., wheat and rye flour extracts) can form gels in the presence of certain oxidants (e.g., upon the addition of hydrogen peroxide).

20       The phenomenon is known in the art as "oxidative gelation", and an extensive literature exists on the subject of oxidative gelation of wheat flour extracts. The term "oxidative cross-linking" is used *mutatis mutandis* to define the oxidative coupling of polymers which is associated with oxidative gelation. The terms "oxidative gelation" and "oxidative cross-linking" are used herein in a broad sense to include the case where viscous solutions are produced rather than true gels, and the term "gel" is therefore to be interpreted loosely to cover viscous liquids. This reflects the fact that oxidative gelation/cross-linking  
25       are progressive phenomena which may be controlled to vary the degree of cross-linking/gelation to the extent that hard, brittle gels are formed at one extreme and slurries or viscous liquids at the other.

30       The biochemical basis of the gelling process is not completely or consistently described in the prior art. According to one model, the gels arise as high molecular weight arabinoxylan and protein molecules become inter- and/or intra-linked (*via inter alia* phenolic substituents, for example ferulic acid-derived diferulate bridges): see e.g., Hosene and Faubion (1981), *Cereal Chem.*, 58:421.

35       In another model, gel formation and/or viscosity increases arise (at least in part) from cross-linking within and/or between macromolecular components of the hemicellulose mediated by ferulic acid residues (for example, involving diferulate generated by oxidative coupling of the aromatic nucleus of ferulic acid).

It should be noted that, as used herein (and as is usual in the art), the terms "ferulic acid" and "ferulate" are used *sensu lato* encompass ferulyl (often denoted feruloyl) groups

(i.e., 4-hydroxy-3-methoxy-cinnamyl groups) and derivatives (particularly oxidized derivatives) thereof.

Only a few oxidizing agents are known to have the ability to induce gelation, and these include hydrogen peroxide (usually in conjunction with a peroxidase), ammonium persulphate and formamidine disulphide.

Most of the work in the area of oxidative gelation has focused on water soluble pentosans from wheat flour. In these studies, wheat flour is extracted with water (usually at room temperature) to yield gelling arabinoxylans. However, water-insoluble wheat pentosans extracted from wheat flours with various concentrations of cold sodium hydroxide have also been shown to form gels (Michniewicz *et alia*, Cereal Chemistry 67(5):434-439 (1990), and oxidative gelation of beet pectins has also been described: see J.-F. Thibault *et alia*, in The Chemistry and Technology of Pectin, Academic Press 1991, Chapter 7, pages 119-133) and FR 2 545 101 A1, discussed earlier.

WO 93/10158 describes the preparation of hemicellulosic material from various brans and the oxidative gelation of maize-derived hemicelluloses using an oxidizing system comprising a peroxide (such as hydrogen peroxide) and an oxygenase (such as a peroxidase). The hemicellulosic material for use as a gelling agent is prepared by hot water or mild alkali extraction.

WO 96/03440 describes the use of an oxidase (preferably a laccase) for promoting oxidative gelation of *inter alia* arabinoxylans. However, laccase may not be acceptable for use in certain food applications, is relatively expensive and the supply is limited. Moreover, oxidases such as laccase are relatively weak oxidation-promoters, and the range of different gel strengths obtainable by the use of such enzymes is limited. Indeed, it is possible that the crosslinking achieved through the use of laccase and other oxidases differs fundamentally from that mediated by e.g., hydrogen peroxide, so that the gels may differ significantly in structure from those produced by other forms of oxidative gelation.

The gels and viscous fluids derived from gelling hemicelluloses (and in particular arabinoxylan and pectin) have long been recognized to have a wide variety of uses in industry (particularly the food industry). There is therefore great interest in the mechanisms which promote the gelling of these materials and in processes for increasing the efficiency of the gelling process and the quality of the resultant gels.

#### SUMMARY OF THE INVENTION

It has now surprisingly been discovered that certain arabinoxylan and pectin polymers interact synergistically when mixtures of these polymers are oxidatively cross-linked: the gelling capacity of the mixtures is far greater than the sum of the contribution of each polymer alone (on a weight/volume basis). The phenomenon is so marked that in some circumstances strong gels can be produced using quantities of mixed polymers which would not gel (or produce only very weak gels) if used alone in similar quantities. The rate of

formation of a gel by the mixture of polymers has been found to be dependent on pH, thus adjustment of pH may be necessary to achieve the desired gelling.

Since the chemical structures of a wide variety of different arabinoxylans and pectins have been exhaustively characterised and described in the prior art, the implications of this discovery extend beyond applications based on naturally-occurring vegetable hemicellulose (e.g., arabinoxylan and/or pectin) extracts: the invention permits the rational design, synthesis and formulation *in vitro* of a novel range of polymer compositions with predetermined and highly desirable gelling characteristics.

According to the present invention there is provided a composition comprising a first polymer comprising arabinosyl-substituted (1-4)- $\beta$ -xylopyranosyl units cross-linked to a second polymer comprising D-galacturonic acid units. A closely related aspect of the invention provides a composition comprising co-gelled first and second polymers, the first polymer comprising arabinosyl-substituted (1-4)- $\beta$ -xylopyranosyl units and the second polymer comprising D-galacturonic acid units.

The first and/or second polymers need not be present in highly purified form, though it is preferred that the first and second polymers be substantially purified. In the case of first and second polymers which are derived from natural sources, the polymers need not be purified to homogeneity, either with respect to other species of macromolecule or carbohydrate present or with respect to other (non-hemicellulosic) components. Indeed, for most applications the first and/or second polymers will comprise a heterogeneous mixture of different polymers, together with contaminating proteinaceous, carbohydrate, cellulosic and fatty materials (often in minor or trace amounts).

When derived or extracted from a natural (often plant or vegetable) source, the polymers for use in the invention are preferably substantially isolated. The term "isolated" is used herein to indicate that the polymer exists in a physical milieu distinct from that in which it occurs in nature. For example, the isolated polymers may be substantially isolated with respect to the complex chemical milieu in which they naturally occur. The absolute level of purity is not critical, and those skilled in the art can readily determine appropriate levels of purity according to the use to which the polymer(s) are to be put.

In many circumstances, the isolated polymer(s) will form part of a composition (for example a more or less crude extract containing other components), buffer system or pharmaceutical excipient, which may for example contain other components (such as and not limited to colouring agents, flavouring agents, antimicrobial agents, enzymes, preservatives or dispersants).

In other circumstances, the isolated polymer(s) may be purified to essential homogeneity. In preferred embodiments, the isolated polymer(s) of the invention are essentially the sole active gelling agents in a given composition.

As explained above, the first and second polymers may be present in a form and at relative and/or absolute concentrations such that they produce a synergistic effect on gel strength or viscosity.

5 The first and second polymers may comprise phenolic acid substituents, the first and second polymers being crosslinked *via* the phenolic acid substituents. Preferably, the phenolic acid substituents comprise ferulic acid substituents. Particularly preferred are compositions wherein the phenolic acid substituents of the first polymer comprise  $\alpha$ -L-arabinofuranosyl residues (as is the case with naturally-occurring arabinoxylans).

10 The cross-links may comprise diferulate bridges, though any other convenient form of covalent crosslinking may be employed. Particularly preferred are compositions in which the first and second polymers are oxidatively crosslinked.

The first polymer may conveniently comprise an arabinoxylan, preferably a gelling arabinoxylan. Particularly preferred is arabinoxylan ferulate. The second polymer may conveniently comprise a pectin, preferably a gelling pectin. Particularly preferred is a  
15 feruloylated pectin.

For many applications, the composition is preferably in the form of a gel or viscous fluid. For other applications the composition may be provided in the non-cross-linked or non-co-gelled state. The latter is preferred for applications where gelation/cross-linking is to be carried out by the end user. In yet other applications, the composition may be provided as  
20 a gel or viscous fluid in dehydrated form. Also contemplated are such dehydrated gels or viscous fluids in rehydrated form.

In another aspect the invention provides a process for preparing a gel or viscous fluid comprising the step of oxidatively co-gelling a first polymer comprising arabinosyl-substituted (1-4)- $\beta$ -xylopyranosyl units and a second polymer comprising D-galacturonic  
25 acid units. Also contemplated are gels or viscous fluids produced by (or obtainable by) the processes of the invention.

The invention also contemplates a pharmaceutical or cosmetic preparation or medical device comprising the composition of the invention, the preparation or device being for example selected from: a wound plug, wound dressing, controlled release device, an  
30 encapsulated medicament or drug, a lotion, cream, suppository, pessary, spray, artificial skin, protective membrane, a nutraceutical, prosthetic, orthopaedic, ocular insert, injectant, lubricant or cell implant matrix, optionally further comprising an antibiotic, analgesic and/or anti-inflammatory agent.

The composition of the invention finds application in therapy, prophylaxis or  
35 diagnosis, for example in the treatment of skin lesions (e.g., burns, abrasions or ulcers).

Also contemplated is a bread improver comprising the composition of the invention as well as a foodstuff, dietary fibre source, food ingredient, additive, lubricant, supplement or dressing comprising the composition of the invention, for example being selected from a



drink (e.g., a stabilized milk-protein drink), yogurt, chocolate, a petfood (wherein the gel e.g., acts as a binder), a preserve (e.g., jam or marmalade), a flavour delivery agent, a stabilizer, a pectin replacer, a canning gel, fat replacer (e.g., comprising macerated gel of any one of the preceding claims), a coating, a glaze, a bait or a gelatin replacer.

5 Also contemplated are masking agents comprising the composition of the invention, for example for use in masking semiconductor wafers, etching plates or surfaces to be painted.

#### BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts the rate of gelation of AXF-sugar beet pectin mixtures at 25°C.  
10 Figure 1 shows G', Pa (elastic modulus) as a function of time, in minutes, for mixtures of AXF with sugar beet pectin. 50 ul peroxidase plus 50 ul peroxide to 50 g of solution. All solutions 2 wt % unless stated otherwise. 1 wt % pectin did not gel.

Figure 2 shows gelation of AXF, SBP and AXF-SBP (1:1) mixture at 2 wt %, pH 4 and 25°C.

15 Figure 3 depicts gelation of AXF, SBP and AXF-SBP (1:1) mixture at 2 wt %, pH 5 and 25°C.

#### DETAILED DESCRIPTION

##### Polymers for use in the invention

The first polymer for use in the invention may be any polymer comprised of  
20 arabinosyl-substituted (1-4)- $\beta$ -xylopyranosyl units. Preferred are arabinoxylans or heteroxylans or their synthetic counterparts (i.e., structural analogues of a naturally-occurring arabinoxylans/heteroxylans synthesised *in vitro* by any chemical/enzymic synthesis or modification). Particularly preferred are arabinoxylans with substituents with phenolic (e.g., ferulic acid) groups which are cross-linkable with certain oxidizing agents.  
25 These "gelling" arabinoxylans are particularly preferred for use in the invention. Thus, of the arabinoxylans, particularly preferred are AXFA and AXF.

The second polymer for use in the invention may be any polymer comprising D-galacturonic acid units. Particularly preferred for use in the invention are gelling (e.g., feruloylated) pectins, including the true pectins, simple pectins, complex pectins and  
30 mesocomplex pectins. Pectins obtainable from representatives of the plant family *Chenopodiaceae*, which include beets (e.g., sugar beet), spinach and mangelwurzel are particularly suitable, and especially preferred is sugar beet pectin (for example in the form of sugar beet pulp). Also useful in the invention are treated pectins (as hereinbefore defined). Also suitable are synthetic pectins (i.e., structural analogues of naturally-occurring pectins  
35 synthesised *in vitro* by any chemical/enzymic synthesis or modification).

The first and/or second polymers may be obtained from a wide range of plant sources. Thus, suitable starting materials containing hemicellulose for use in the processes of the invention (either as starting materials in the fractionation processes or as sources of

hemicellulose *per se*) typically include plant material of various kinds and any part or component thereof.

Plant materials useful as a starting material in the invention include the leaves and stalks of woody and nonwoody plants (particularly monocotyledonous plants), and grassy  
5 species of the family Gramineae. Particularly preferred are gramineous agricultural residues, i.e., the portions of grain-bearing grassy plants which remain after harvesting the seed. Such residues include straws (e.g., wheat, oat, rice, barley, rye, buckwheat and flax straws), corn stalks, corn cobs and corn husks.

Other suitable starting materials include grasses, such as prairie grasses, gamagrass  
10 and foxtail. Other suitable sources include dicotyledonous plants such as woody dicots (e.g., trees and shrubs) as well as leguminous plants.

Another preferred source are fruits, roots and tubers (used herein in the botanical sense). The term "fruit" includes the ripened plant ovary (or group thereof) containing the seeds, together with any adjacent parts that may be fused with it at maturity. The term  
15 "fruit" also embraces simple dry fruits (follicles, legumes, capsules, achenes, grains, samaras and nuts (including chestnuts, water chestnuts, horsechestnuts etc.)), simple fleshy fruits (berries, drupes, false berries and pomes), aggregate fruits and multiple fruits. The term "fruit" is also intended to embrace any residual or modified leaf and flower parts which contain or are attached to the fruit (such as a bract). Encompassed within this meaning of  
20 fruit are cereal grains and other seeds.

Also contemplated for use as starting materials are fruit components, including bran, seed hulls and culms, including malt culms. "Bran" is a component of cereals and is defined as a fraction obtained during the processing of cereal grain seeds and comprises the  
25 lignocellulosic seed coat as separate from the flour or meal. Other suitable component parts suitable as starting materials include flours and meals (particularly cereal flours and meals, and including nonwoody seed hulls, such as the bracts of oats and rice).

The term "root" is intended to define the usually underground portion of a plant body that functions as an organ of absorption, aeration and/or food storage or as a means of anchorage or support. It differs from the stem in lacking nodes, buds and leaves. The term  
30 "tuber" is defined as a much enlarged portion of subterranean stem (stolon) provided with buds on the sides and tips.

Preferred lignocellulosic starting materials include waste stream components from commercial processing of crop materials such as various beets and pulps thereof (including sugar beet pulp), citrus fruit pulp, wood pulp, fruit rinds, nonwoody seed hulls and cereal  
35 bran. Suitable cereal sources include maize, barley, wheat, oats, rice, other sources include pulses (e.g., soya), legumes and fruit.

Other suitable starting materials include pollen, bark, wood shavings, aquatic plants, marine plants (including algae), exudates, cultured tissue, synthetic gums, pectins and mucilages.

5 Particularly preferred as a starting material for the first polymer is testaceous plant material, for example waste testaceous plant material (preferably containing at least about 20% of arabinoxylan and/or glucoronoarabinoxylan).

The starting material may be treated directly in its field-harvested state or (more usually) subject to some form of pre-processing. Typical pre-processing steps include chopping, grinding, cleaning, washing, screening, sieving, etc.

10 Preferably, the starting material is in a substantially ground form. It may be air classified or sieved (for example to reduce the level of starch). Alternatively, or in addition, the starting material may be treated with enzymes to remove starch (e.g., alpha- and/or beta-amylase). The starting material may also be pre-digested with a carbohydrase enzyme to remove  $\beta$ -glucan.

15 Suitable washing treatments include washing with hot water or acid (e.g., at a pH of 3-6, e.g., about 5). This at least partially separates protein. Other pre-treatments include protease treatment.

#### Post-extraction processing/isolation

20 Once extracted and prior to oxidative gelation, the polymer(s) may be further processed to concentrate, purify or simply isolate them from the unextracted residue.

Other post-extraction treatments include supplementing the extracted polymer(s) with an oxidase (e.g., glucose oxidase) supplement, optionally together with a peroxidase (e.g., horse radish peroxidase) and/or an oxidase substrate (e.g., glucose) supplement. This supplementing step is carried out when gelation is to be carried out subsequently by *in situ* generation of hydrogen oxide by redox enzymes.

25 Particularly preferred are post-extraction processes which avoid the use of alcohol precipitation, so avoiding the costs associated with this step.

Preferred processing steps include any of centrifugation, filtration (e.g., ultrafiltration or filtration of vega clay), precipitation (e.g., isoelectric precipitation), chromatography (e.g., silica hydrogel and/or ion exchange chromatography). Particularly preferred is ultrafiltration or concentration by spray-, drum- or freeze-drying, or vacuum rotary drying. Other treatments include desalting treatments, for example dialysis or tangential flow ultrafiltration.

35 Although not preferred, alcohol (e.g., IMS, methanol, ethanol or iso-propanol) precipitation, for example with up to 60-70% v/v alcohol, may be employed. However, particularly preferred is direct spray or freeze drying followed by drying, in the absence of an alcohol precipitation step.

Any of the aforementioned processes may be applied directly to the extracted polymer(s). The extract may be dried, either before or after oxidative gelation. Dried preparations may be supplemented with carriers or dispersants, such as glucose.

#### Oxidative gelation

5 Any of a variety of known oxidative gelation process can be used to gel the polymers of the invention. Suitable agents include hydrogen peroxide (usually in conjunction with a peroxidase), ammonium persulphate and formamidine disulphide.

The oxidative gelation may also be accomplished enzymically, for example as described in WO 96/03440 in which an oxidase (preferably a laccase) is used to promote  
10 oxidative gelation of *inter alia* arabinoxylans.

Other enzymic approaches include promoting the generation of hydrogen peroxide *in situ* by redox enzymes. The redox enzymes preferably comprise an oxidase (e.g., glucose oxidase) and a peroxidase (e.g., horse radish peroxidase), which are preferably present as supplements in the hemicellulosic material.

15 Alternatively, gelation may be achieved as described in WO 93/10158, which describes an oxidizing system comprising a peroxide (such as hydrogen peroxide) and an oxygenase (such as a peroxidase).

Gelation of the first and second polymers of the invention is carried out at a gelling pH, that is a pH at which coupling of the polymers takes place to form a gel or viscous fluid.  
20 A suitable pH is 9 or below, for example 8 or below. The gelling pH may be from 4 to 8. Preferably the first and second polymers are co-gelled at a pH of 5 or below. The desired gelling pH may be achieved by adjusting the pH of the biopolymer solution using small amounts of dilute alkali or acid, for example sodium hydroxide or hydrochloric acid.

#### Applications

25 The compositions of the invention (i.e., the gels, dehydrated gels, rehydrated dehydrated gels, gelling (but un-gelled) compositions and viscous liquids of the invention) find a variety of applications in various therapeutic, surgical, prophylactic, diagnostic and cosmetic (e.g., skin care) applications.

For example, the aforementioned materials may be formulated as a pharmaceutical or  
30 cosmetic preparation or medical device, for example selected from: a wound plug, wound dressing, wound debriding system, controlled release device, an encapsulated medicament or drug, a lotion, cream (e.g., face cream), suppository, pessary, spray, artificial skin, protective membrane, a nutraceutical, prosthetic, orthopaedic, ocular insert, injectant, lubricant or cell implant matrix. They may be particularly useful as agents which maintain the integrity of  
35 the gut wall lining, and as agents for coating the luminal wall of the gastrointestinal tract. They may therefore find particular application in animal feeds and in the treatment of gastrointestinal disorders.

In such embodiments the compositions of the invention may further comprise an antibiotic, electrolyte, cell, tissue, cell extract, pigment, dye, radioisotope, label, imaging agent, enzyme, co-factor, hormone, cytokine, vaccine, growth factor, protein (e.g., a therapeutic protein), allergen, hapten or antigen (for e.g., sensitivity testing), antibody, oil, analgesic and/or anti inflammatory agent (e.g., NSAID).

Thus, the above-listed materials find application in therapy, surgery, prophylaxis or diagnosis, for example in the treatment of surface (e.g., skin or membrane lesions, e.g., burns, abrasions or ulcers). In a particularly preferred embodiment, the invention contemplates a wound dressing comprising the above listed materials of the invention, for example in the form of a spray. Such wound dressings are particularly useful for the treatment of burns, where their great moisture retaining properties help to prevent the wound drying out.

Particularly preferred for such application is a self-gelling liquid comprising the composition of the invention supplemented with glucose and peroxidase and/or oxidase enzymes which gels on contact with oxygen in the air. Such compositions can be provided in the form of oxygen-free liquids in airtight containers which can be sprayed onto the skin, whereupon the liquid gels after exposure to the air. Such composition may advantageously be formulated so as to produce a slight excess of hydrogen peroxide on exposure to oxygen, so that a sterilizing, antibacterial, bacteriostatic and/or cleansing effect is obtained which helps promote healing.

The invention also contemplates water absorbent nappies, diapers, incontinence pads, sanitary towels, tampons and panty liners comprising the above-listed materials, as well as domestic and industrial cleaning or liquid (e.g., water) recovery operations (e.g., in the oil industry).

Alternatively, the gels of the invention can be provided in the form of hydrated or dehydrated sheets or pellicles for application to various internal or external surfaces of the body, for example during abdominal surgery to prevent adhesions.

Other applications include enzyme immobilizing systems, brewing adjuncts and bread improvers.

The materials listed above also find application as a foodstuff, dietary fibre source, food ingredient, additive, lubricant, supplement or food dressing. Such products are preferably selected from crumb, alginate replacer, cottage cheeses, aerosol toppings, frozen yoghurts, milk shakes, ice cream, low calorie products such as dressings and jellies, batters, cake mixes, frozen chips, binders, gravies, pastas, noodles, doughs, pizza toppings, sauces, mayonnaise, jam, preserve, pickles, relish, fruit drinks, a clouding agent in drinks, syrups, toppings and confectionary (e.g., soft centres), petfood (wherein the gel e.g., acts as a binder), a flavour delivery agent, a canning gel, fat replacer (e.g., comprising macerated gel), a coating, a glaze, a bait, a binder in meat and meat analogue products (for example

vegetarian products), an edible adhesive, a gelatin replacer or dairy product or ingredient (e.g., a yoghurt supplement).

When used as a fat replacer the gel of the invention is preferably macerated to optimize its mouthfeel and fat mimetic properties.

- 5       The invention will now be further illustrated by way of specific Examples, which are purely illustrative and not intended to limit the scope of the invention in any way.

#### EXAMPLE 1

##### Production of arabinoxylan ferulate

- 10       10 l of sodium acetate buffer (pH 5, 0.02 M) were pre-equilibrated at 50°C and 10 ml of liquid protease (Profix™) was added.

1 kg of fine wheat bran was added to this enzyme solution, and the suspension mixed vigorously for 60 min, maintaining the temperature at 50°C. The bran residue was then washed over a 200 µm sieve, and rinsed with 3 l of hot water. The washings were discarded and the bran residue recovered.

- 15       The washed bran residue was then resuspended in 5 l of sodium acetate buffer (pH 5, 0.02 M) at 60°C and mixed continuously, maintaining the temperature at 60°C. 25 g of KOH pellets were then added, and mixing continued for 60 min at 60°C.

- 20       After 60 min, the mixture was neutralized to pH 7 with acetic acid and filtered to recover liquid. The mixture was then left to stand while a precipitate forms. Alternatively, the mixture may be centrifuged. A clear, dark golden brown supernatant is recovered.

The pH of the supernatant was then brought to pH 4.8 with acetic acid and 1.5 volumes of IMS added. Further acetic acid is added to maintain the pH at 4.8.

Polysaccharides are then recovered by centrifugation and solvent exchange, and the polysaccharides then dried with acetone.

#### 25       Gelation

- Solutions of hydrocolloids were prepared by gradually adding dry powder to water with stirring until completely dispersed. Once prepared, solutions were stored at 4°C until required (two days maximum). AXF or sugar beet pectin (supplied by Copenhagen Pectin) were placed in plastic containers to give a total solution weight of 50 g. No adjustments were made for pH or ionic environment. Gelation was commenced by adding peroxidase followed by peroxide solution. As soon as the peroxide was added the container was quickly shaken to mix the reactants and a portion of the sample placed on the rheometer. There was a delay of approximately 1 minute between the start of the reaction and first measurement. Gelation was followed in oscillatory dynamic mode using a strain of 1% and frequency of 1 rads<sup>-1</sup>. Parallel plate geometry was used with 50 mm diameter plates and a gap of 1 mm. Measurements were taken every 30 s for 45 min, 60 min or 120 min. The temperature was maintained at 25°C. In some instances gelled systems were prepared as above and stored at ambient temperature overnight for visual assessment the next day.

### Results and discussion

The minimum concentration for gelation of the AXF alone was approximately 0.25 wt %. Figure 1 shows  $G'$  (elastic modulus) as a function of time, in minutes, for mixtures of AXF with sugar beet pectin. Sugar beet pectin alone at 2 wt % gave the smallest increase in  $G'$  and did not gel at all at 1 wt %. However, the rate of reaction of sugar beet pectin was faster than that of AXF and most of the increase in  $G'$  was complete within 5 min. The addition of AXF to sugar beet pectin resulted in an increase in  $G'$ ; for example,  $G'$  (60 min) for 2 wt % pectin was 97 Pa and for AXF:pectin 0.25:0.75 (2 wt % total) was 514 Pa. The highest values of  $G'$  (after 60 min) were for AXF:sugar beet pectin mixtures of the ratios 0.9:0.1, 0.8:0.2 and 0.75:0.25. Gelation of all mixtures containing sugar beet pectin was faster than for AXF alone.

To investigate whether the synergistic effect of sugar beet pectin-AXF mixtures was due to effects other than cross-linking through ferulic acid residues, the reaction was done with a mixture of pectin with non-gelling arabinoxylan (AX). The synergistic effect was not apparent with this form of arabinoxylan, showing that the increase in  $G'$  for the gelation of sugar beet pectin is not a salt or non-electrolyte effect.

#### EXAMPLE 2

##### Effect of pH on the rate of gel formation

The rate of gel formation, ie. increase in the elastic modulus, ( $G'$ ) as a function of time, was measured following the adjustment of the pH of solutions of AXF, sugar-beet pectin (SBP) and AXF-SBP (1:1) by the addition of small amounts of dilute NaOH or HCl. In all cases the final concentration of biopolymer was 2 wt %.

Figures 2 and 3 show gel formation for AXF, SBP and AXF-SBP (1:1) mixtures at pH 4 and 5 respectively. The 4th line (AXF-SBP-calc) is the average of the AXF and SBP curves. Both Figures 2 and 3 show that the measured curve deviates from the predicted curve; measured values of  $G'$  being greater than predicted, demonstrating a synergistic effect.

Various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

The disclosure of each reference set forth above is incorporated herein by reference in its entirety.

CLAIMS

What is claimed is:

1. A composition comprising a first polymer comprising arabinosyl-substituted (1-4)- $\beta$ -xylopyranosyl units cross-linked to a second polymer comprising D-galacturonic acid units.
2. A composition comprising co-gelled first and second polymers, the first polymer comprising arabinosyl-substituted (1-4)- $\beta$ -xylopyranosyl units and the second polymer comprising D-galacturonic acid units.
3. The composition of Claim 1 or Claim 2 wherein the first and second polymers comprise phenolic acid substituents, the first and second polymers being crosslinked via phenolic acid substituents.
4. The composition of Claim 3 wherein the phenolic acid comprises ferulic acid.
5. The composition of Claim 3 wherein the phenolic acid substituents of the first polymer comprise  $\alpha$ -L-arabinofuranosyl residues.
6. The composition of Claim 4 or Claim 5 wherein said polymers are crosslinked via diferulate bridges.
7. The composition of any one of the preceding claims wherein the first and second polymers are oxidatively crosslinked.
8. The composition of any one of the preceding claims wherein the first polymer comprises an arabinoxylan.
9. The composition of Claim 8 wherein the arabinoxylan comprises a gelling arabinoxylan.
10. The composition of Claim 9 wherein the gelling arabinoxylan comprises arabinoxylan ferulate.
11. The composition of any one of the preceding claims wherein the second polymer comprises a pectin.
12. The composition of Claim 11 wherein the pectin comprises a gelling pectin.
13. The composition of Claim 12 wherein the gelling pectin comprises a feruloylated pectin.
14. The composition of any one of the preceding claims wherein the composition is in the form of a gel or viscous fluid.
15. The composition of any one of the preceding claims wherein the first and second polymers are co-gelled at a gelling pH.
16. The composition of Claim 15 wherein the gelling pH is 9 or below.
17. The composition of Claim 16 wherein the gelling pH is from 4 to 8.
18. The composition of any one of Claims 14 to 17 wherein the first and second polymers produce a synergistic effect on gel strength or viscosity.



19. The composition of Claim 18 wherein the first and second polymers are co-gelled at a gelling pH of 5 or below.

20. The composition of any one of the preceding claims in the non-cross-linked or non-co-gelled state.

5 21. The composition of Claim 14 wherein the gel or viscous fluid is in dehydrated form.

22. The composition of Claim 21 wherein the gel or viscous fluid is in rehydrated form.

10 23. A process for preparing a gel or viscous fluid comprising the step of oxidatively co-gelling a first and second polymer as defined in any one of Claims 1-13.

24. The process of Claim 23 wherein the first and second polymers are co-gelled at a gelling pH.

25. The process of Claim 24 wherein the gelling pH is 9 or below.

26. A gel or viscous fluid produced by the process of any one of Claims 23-25.

15 27. A pharmaceutical or cosmetic preparation or medical device comprising the composition of any one of Claims 1-22.

28. The preparation or device of Claim 27 selected from the group consisting of a wound plug, wound dressing, controlled release device, an encapsulated medicament or drug, a lotion, cream, suppository, pessary, spray, artificial skin, protective membrane, a  
20 nutraceutical, prosthetic, orthopaedic, ocular insert, injectant, lubricant and cell implant matrix, optionally further comprising an antibiotic, analgesic, anti-inflammatory agent, and mixtures thereof.

29. The composition of any one of the Claims 1-22 for use in therapy, prophylaxis or diagnosis.

25 30. A bread improver comprising the composition of any one of Claims 1-22.

31. A foodstuff, dietary fibre source, food ingredient, additive, lubricant, supplement or dressing comprising the composition of any one of Claims 1-22.

32. A foodstuff, dietary fibre source, food ingredient, additive, lubricant, supplement or dressing of Claim 31 selected from the group consisting of a drink, yogurt, chocolate, a  
30 petfood, a preserve, a flavour delivery agent, a stabilizer, a pectin replacer, a canning gel, fat replacer, a coating, a glaze, a bait and a gelatin replacer.

33. A masking agent comprising the composition of any one of Claims 1-22.

34. The masking agent of Claim 33 for use in masking semiconductor wafers, etching plates or surfaces to be painted.

35

Figure 1

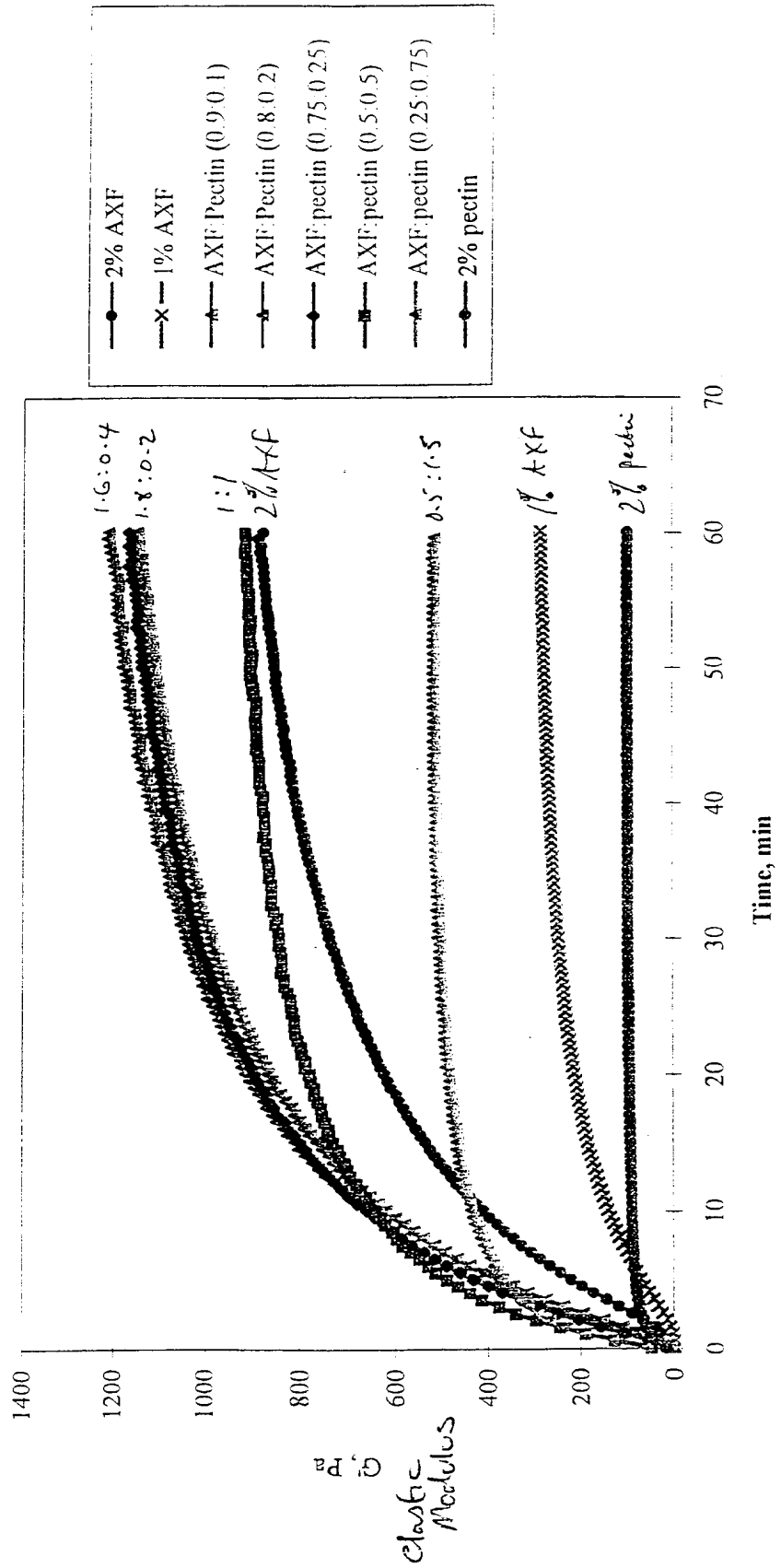


Figure 2

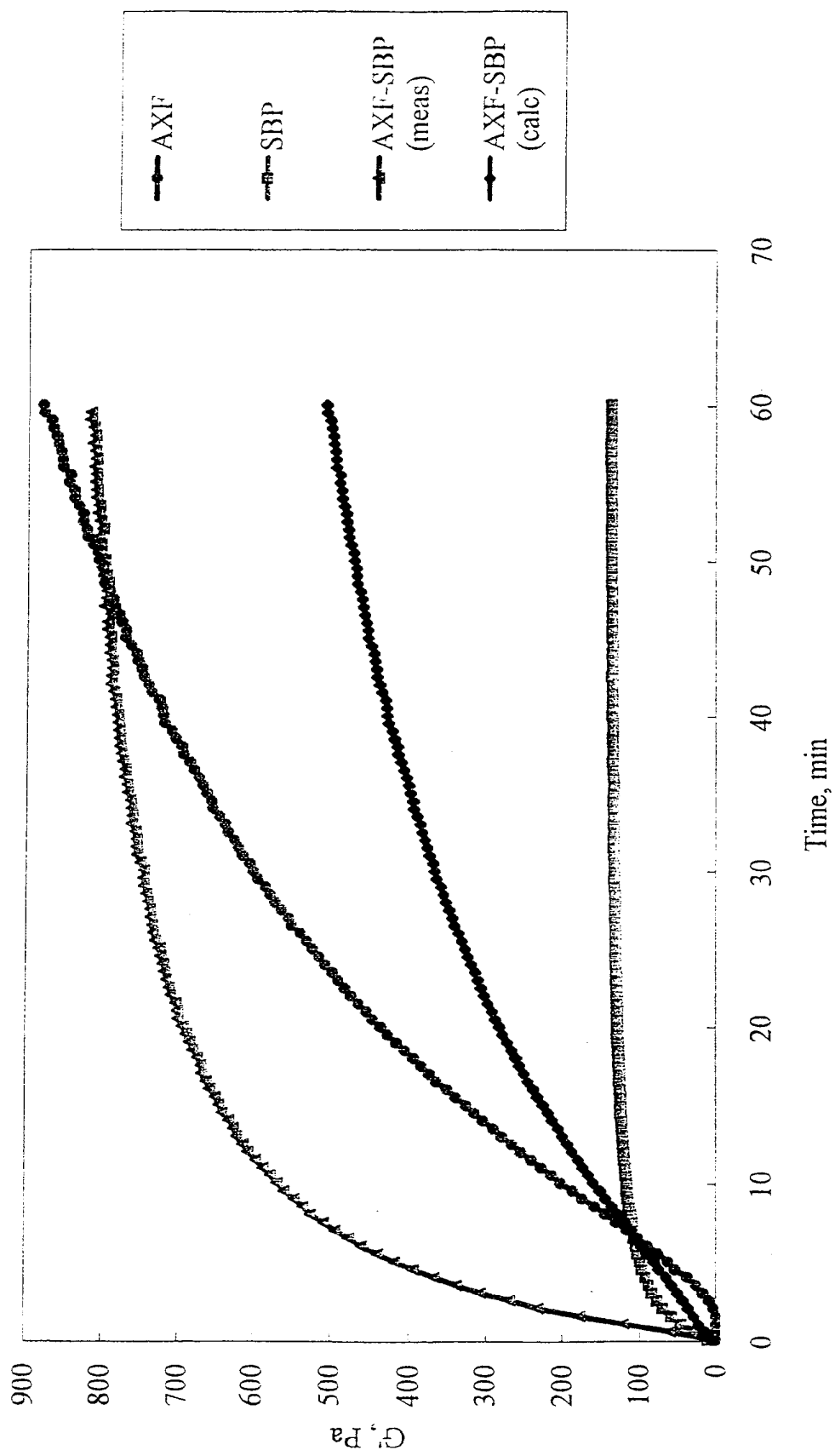
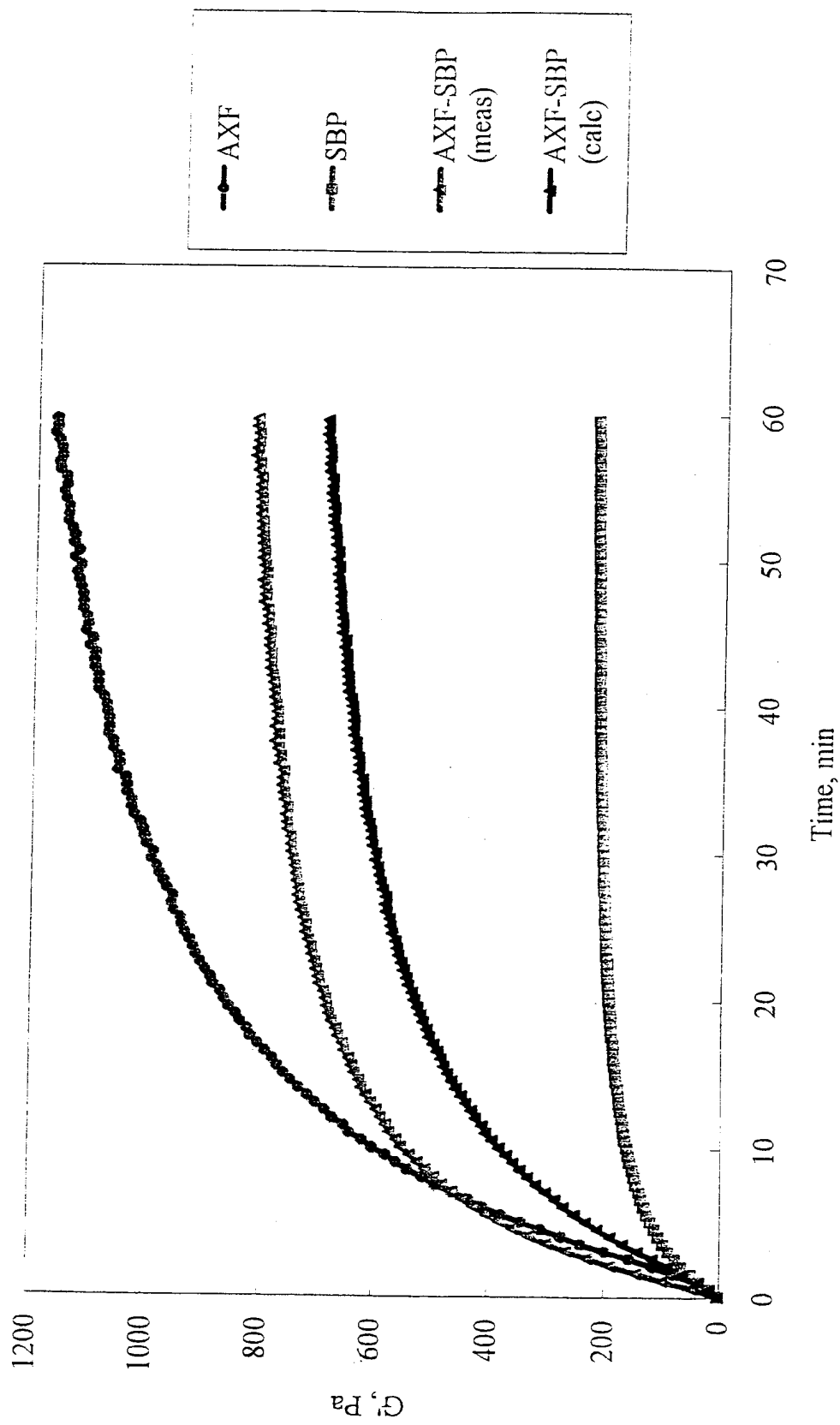


Figure 3



# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/05467

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C08L5/06 C08L5/14 C08B37/00 C08B37/14 C08B37/06  
A61L15/60 A21D2/36 A23L1/052

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C08L C08B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, WPI Data, PAJ

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 03440 A (NOVO NORDISK A/S) 8 February 1996 (1996-02-08) cited in the application  page 6 -page 7 claims 1,12,13	1-3,7-9, 11,12, 14,18, 21-23, 26-32
A	WO 98 22513 A (DALGETY PLC) 28 May 1998 (1998-05-28) claims  -/--	1-12,14, 23,26-34



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

\* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search

4 July 2000

Date of mailing of the international search report

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# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/05467

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	<p>SCHOONEVELD-BERGMANS M E F ET AL:  "Studies on the oxidative cross-linking of  feruloylated arabinoxylans from wheat  flour and wheat bran"  CARBOHYDRATE POLYMERS, GB, APPLIED SCIENCE  PUBLISHERS LTD. BARKING,  vol. 38, no. 4, April 1999 (1999-04),  pages 309-317, XP004160569  ISSN: 0144-8617  abstract  page 316, left-hand column, line 9 - line  37</p>	1-14, 18, 23, 26
A	<p>-----</p> <p>L. SAULNIER &amp; J-F THIBAUT: "Review:  Ferulic acid and diferulic acids as  components of sugar-beet pectins and maize  bran heteroxylans"  JOURNAL OF THE SCIENCE OF FOOD AND  AGRICULTURE,  vol. 79, 1999, pages 396-402, XP002140376  abstract</p> <p>-----</p>	1-14

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/05467

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9603440 A	08-02-1996	AU 3074595 A BR 9508350 A CA 2195640 A CZ 9700205 A EP 0750641 A FI 970274 A HU 77999 A JP 10502962 T	22-02-1996 04-11-1997 08-02-1996 14-05-1997 02-01-1997 23-01-1997 28-04-1999 17-03-1998
WO 9822513 A	28-05-1998	AU 4958997 A EP 0939773 A	10-06-1998 08-09-1999